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13. ABSTRACT (Maximum 200 Words)

Our goal is to understand the role of a novel epithelium-specific Ets transcription factor, PDEF, in prostate cancer. Our progress over the last year has provided significant further evidence that PDEF is an important player in prostate and breast cancer. We were able to characterize the mouse PDEF gene and promoter and to generate knockout constructs for PDEF. We, furthermore, demonstrated that PDEF is a target for kinases in the MAP kinase pathway. We also identified a variety of target genes for PDEF related to adhesion, migration and cell cycle using transcriptional profiling, real time PCR and siRNA interference which will further help us to understand the biological function of PDEF. Our results as well as the critical roles of other Ets factors in cellular differentiation and tumorigenesis strongly suggest that PDEF is an important regulator of prostate gland development and plays a role in prostate and breast cancer progression or development. The new data have further strengthened our belief that PDEF is a prime target for drug development.

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function of a new transcription factor in prostate cancer, gene regulation, epithelial cell differentiation, prostate epithelial cell transformation, cDNA microarrays, bioinformatics, nude mice, tumor formation, proliferation, transgenic mice

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a. Introduction

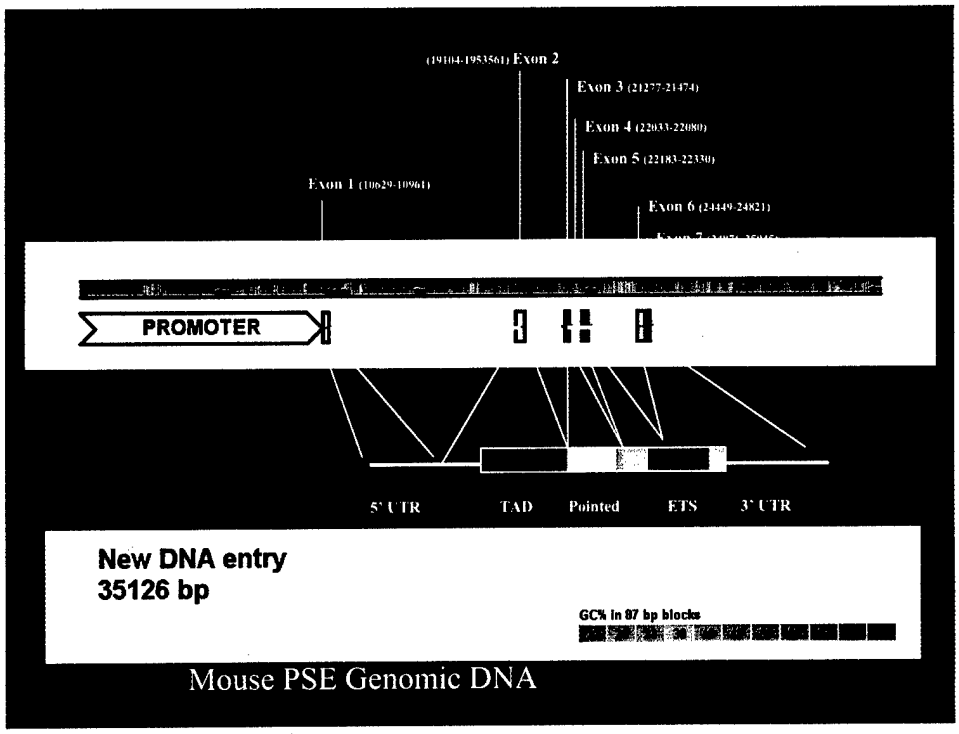
Prostate cancer has become the most common solid cancer in older men and is one of the most frequent causes of cancer deaths. Although androgen ablation therapy, surgery and radiation therapy are effective for the treatment of local prostate cancer, there is no effective treatment available for patients with metastatic androgen-independent disease. The poor prognosis for androgen-independent advanced prostate cancer reflects in part the lack of knowledge about the tumor's basic biology, although progress has been made in identifying defects of various oncogenes and tumor suppressor genes. In particular, very little is known about the molecular mechanisms that trigger the conversion of an initially androgen-dependent cancer to androgen-independence. Our goal is to understand the role of a novel prostate epithelium-specific transcription factor, PDEF, a member of the Ets transcription factor/oncogene family in human prostate cancer that uniquely among the Ets family prefers binding to a GGAT rather than a GGAA core. PDEF is expressed in the luminal epithelial cells of normal human prostate and PDEF expression is significantly elevated in cancerous portions of the prostate. PDEF acts as an androgen-independent transcriptional activator of the PSA promoter, a diagnostic marker used for monitoring androgen-dependent and –independent prostate cancer. PDEF also directly interacts with the DNA binding domain of the androgen receptor and with the prostate-specific homeobox gene NKX3.1 and enhances androgen-mediated activation of the PSA promoter. Thus, our hypothesis is that PDEF bypasses or activates the androgen receptor and thereby contributes to the progression from an initially androgen-dependent prostate cancer to an androgen-independent cancer. We propose to determine the role of this novel member of the Ets family in the conversion of prostate cancer to androgen independence. Our results as well as the critical roles of other Ets factors in cellular differentiation and tumorigenesis strongly suggest that PDEF is an important regulator of prostate gland development and plays a role in prostate epithelial cell transformation and/or prostate cancer progression. Our long term goal is to explore the possibility to use this new factor as another diagnostic tool and as a potential therapeutic target for prostate cancer.

b. Body

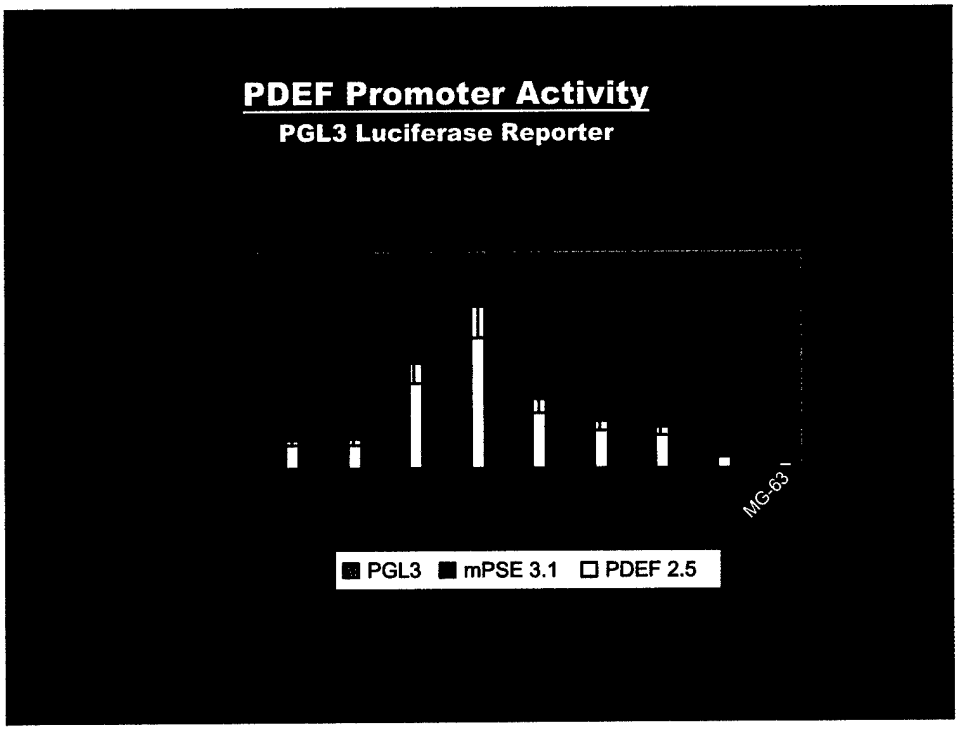
In the last year we made significant progress in several specific aims. Following is a summary of the progress made during this funding period.

Genomic organization of the mouse PDEF gene and generation of knockout constructs

We had previously characterized the human genomic structure of the PDEF gene. We have now sequenced and characterized the murine PDEF gene. The intron/exon structure is very similar to the human gene. We have also sequenced the mouse PDEF promoter sequence and subcloned a 3.1 kb fragment into the luciferase reporter vector. We have started to analyze the activity of the human and mouse PDEF promoter. Most of the activity is present in cells that express endogenous PDEF such as prostate and breast cancer cell lines, whereas PDEF negative cells such as MG-63 osteosarcoma cells and 293 cells express very little PDEF promoter activity. In order to knock out the PDEF gene in mice for studying the effect of PDEF on prostate and mammary gland development, we have generated a construct that deletes the Ets DNA binding domain and the Pointed domain. We have successfully introduced this construct into ES cells and after selection in G418 obtained various neomycin resistant clones. We are now in the process of analyzing these clones by Southern blot hybridization for homologous recombination that deleted the PDEF gene. If success ful, we will generate knockout mice within the next few months.



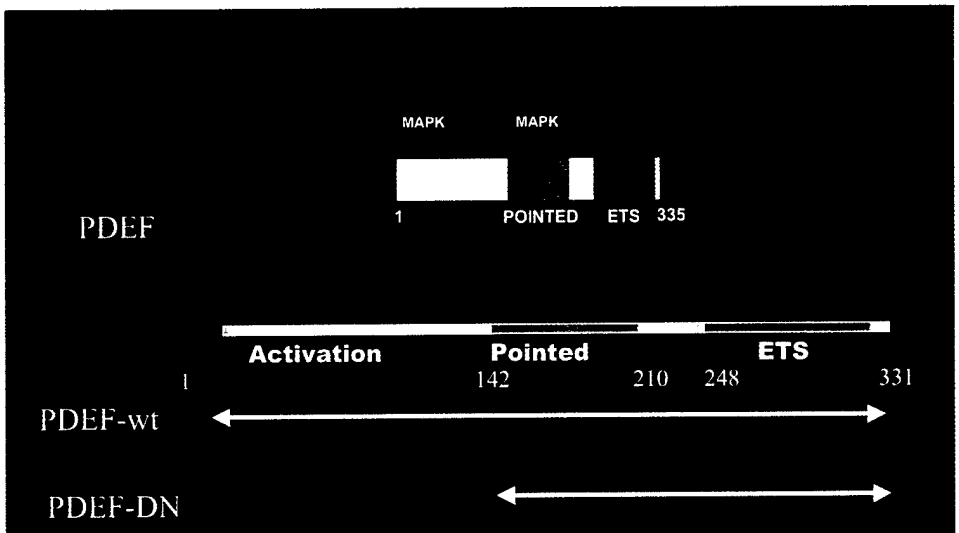
identification of a functional promoter



Characterization of PDEF target genes by oligonucleotide microarrays

We have used Affymetrix oligonucleotide microarrays to determine target genes for PDEF. LNCaP prostate cancer cells as well as SKBR3 breast cancer cells were infected for different times with adenoviruses encoding wild type or dominant-negative mutant PDEF or as control the beta-gal adenovirus vector. RNA was harvested from duplicate experiments 24, 48 and 72 hours after infection and analyzed by hybridization to Affymetrix HU133A chips that contain

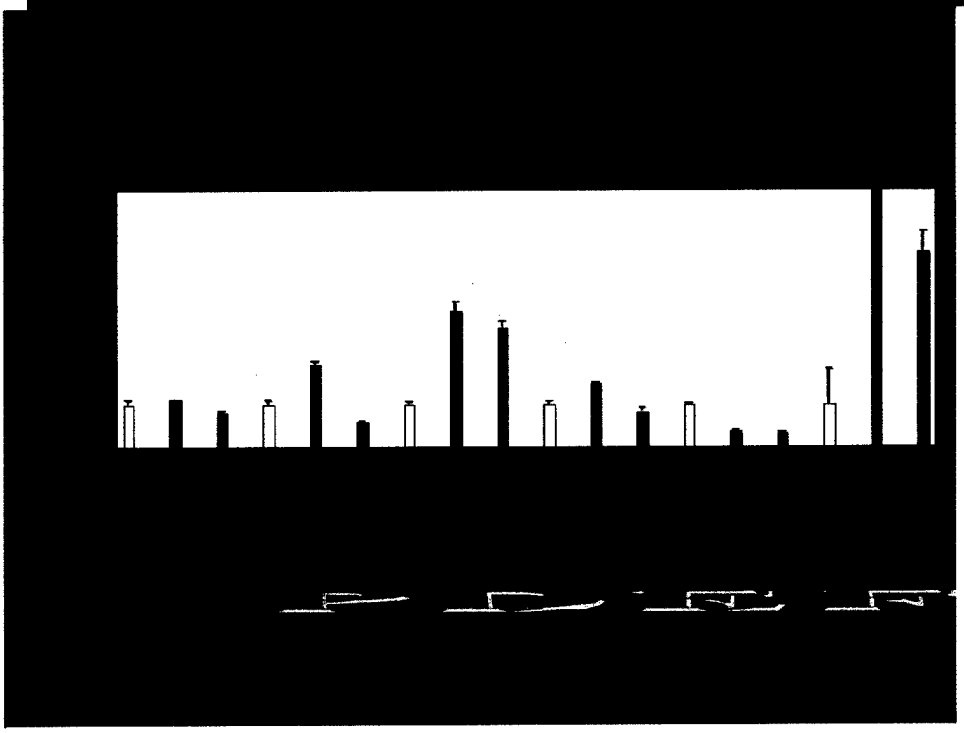
~22000 human genes. Using sophisticated novel bioinformatics tools we have obtained a number of genes whose expression is significantly modulated by PDEF. Various cell cycle, growth, metabolism, adhesion and migration related genes are regulated by PDEF. We have validated at least 30 of these targets by real-time PCR. We have also synthesized siRNA against PDEf in order to inhibit endogenus PDEF expression. PDEF siRNA inhibited PDEF expression on the RNA and the protein level by 50 –80% in LNCaP and SKBR3 cells. Using siRNA we demonstarted that genes that are upregulated by PDEF are downregulated by siRNA and genes that are downregulated by overexpression of PDEF become upregulated by siRNA. Interestingly, the PDEF gene itself is a target for autoregulation by PDEF.

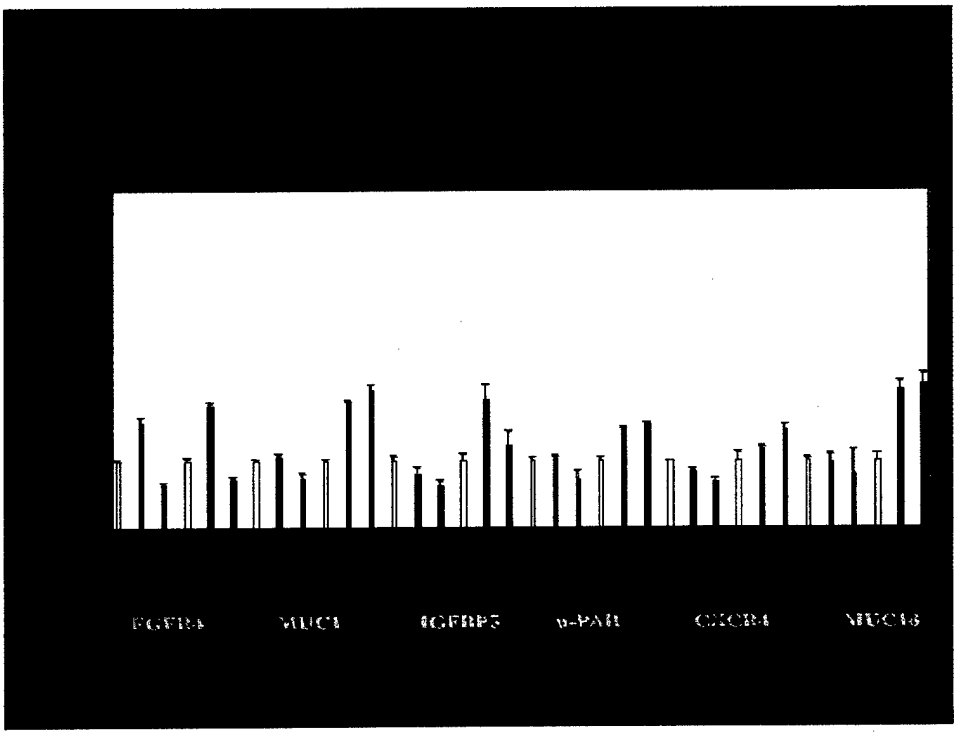


Adeno-X Expression System (Clontech) are used to construct the adenovirus expressed beta-gal (control), PDEF-wt, PDEF-DN

Potential Targets:

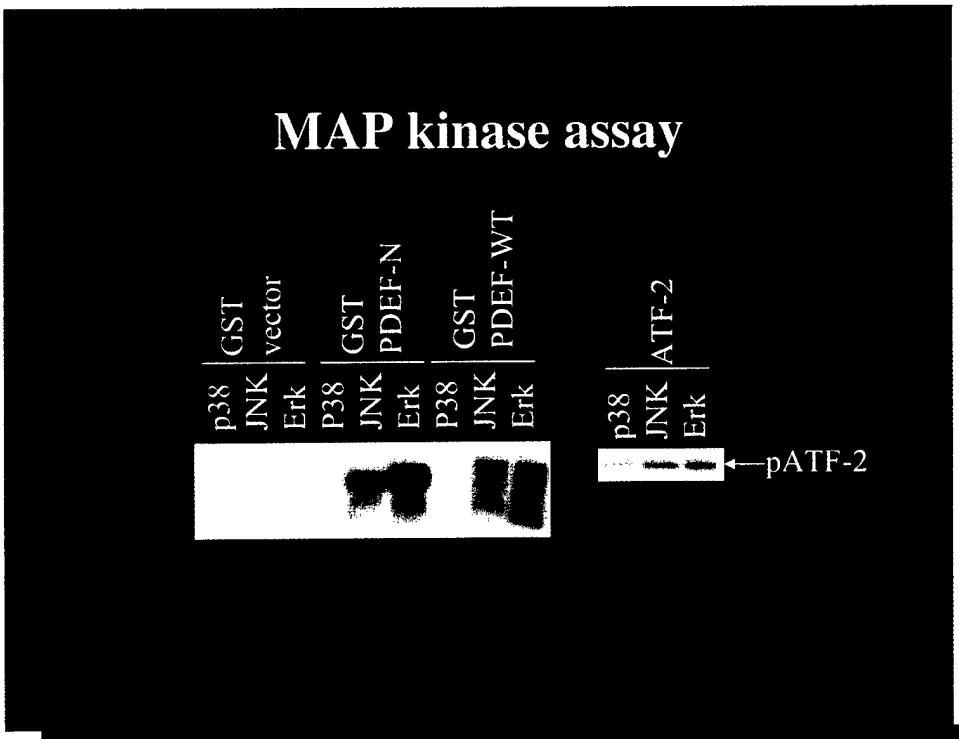
Cell adhesion proteins	
	Febronectin
	clooagen
	Cadeherin
	Integrin
cell cycle proteins	Cyclin G1
	p21
	Cyclin D1
	c/EBP
Growth Factors	Transforming growth factor beta 3
	Ephrin-b1
	Ephrin-b2
	FGFR4
	Insulin-like growth factor binding factor 5
	PDGF (Platelet dereived growth factor)
Membrane receptors	u-PAR
	ErbB1
	CXCR4
Transcription factors	Ezh
	ELF4
	PDEF
Structural proteins	Filamin
	tight junction molecular
	Adhesion molecular
Metabolism process	Fatty acid hydroxylase
	2,3-bisphosphoglycerate mutase
	hydroxysteroid (17-beta) hydrogenase 3

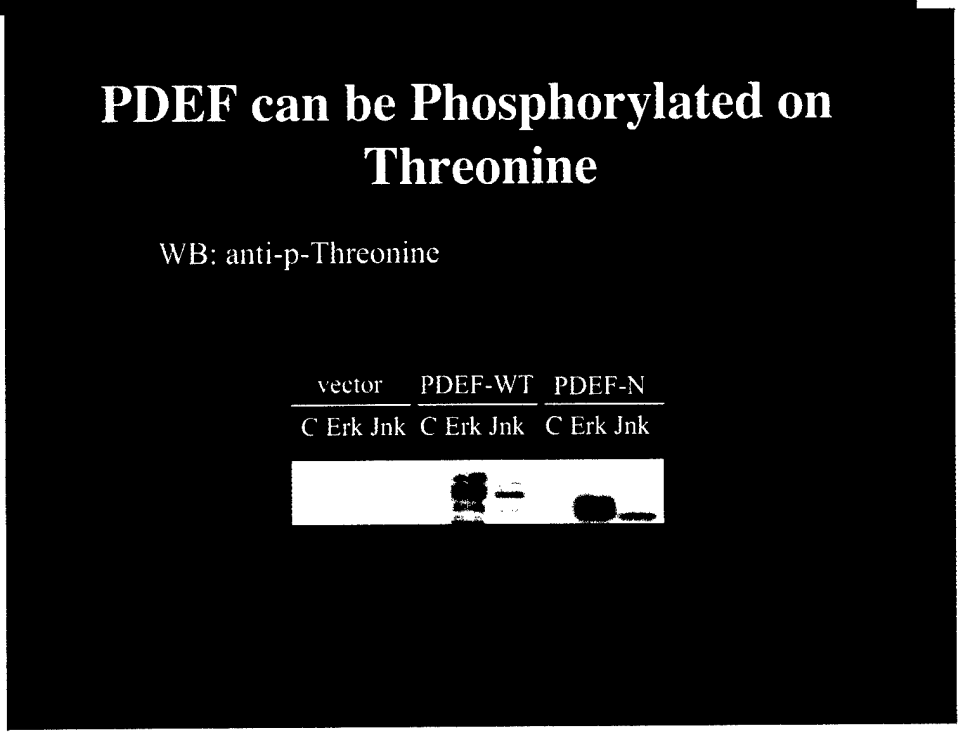




PDEF is a target for Jnk and ERK kinases

The PDEF protein has various putative MAP kinase phosphorylation sites at the amino-terminal transactivation domain. In order to determine whether PDEF may be a target for MAP kinases we performed in vitro kinase assays with FLAG-tagged PDEF and ERK, JNK o rp38 kinases. Both ERK and JNK strongly phosphorylated PDEF indicating that PDEF can indeed be phosphorylated by MAP kinases. To further detail the phosphorylation sites we performed Western blot analysis with anti-phosphothreonine antibodies. This antibody specifically detected PDEF only after phosphorylation by ERK or JNK suggesting that at least some of the phosphorylated residues in PDEF are threonines. We have extended our study to determine whether JNK and ERK can phosphorylate PDEF in vivo and our preliminary data suggest that PDEF indeed gets phosphorylated by these kinases in vivo. We are now in the process to confirm these results and to evaluate the functional consequences of PDEF phosphorylation.





c. Key Research Accomplishments

- Chromosomal mapping and genomic organization of the mouse PDEF gene and identification of a functional promoter
- Generation of PDEF knockout vector and transfection into ES cells
- Identification and validation of target genes for PDEF by transcriptional profiling, real time PCR and siRNA interference
- PDEF phosphorylation by JNK and ERK MAP kinases

d. Reportable Outcomes

Adenoviral vectors for wild type and dominant-negative PDEF
SiRNA for PDEF

e. Conclusions

Alterations in gene expression are central to development and differentiation of tissues, cell death, proliferation and transformation, and in the context of this grant to prostate cancer and the role of PDEF. Our progress over the last year has provided significant further evidence that PDEF is an important player in prostate and breast cancer. We were able to characterize the mouse PDEF gene and promoter and to generate knockout constructs for PDEF. We, furthermore, demonstrated that PDEF is a target for kinases in the MAP kinase pathway. We also identified a variety of target genes for PDEF related to adhesion, migration and cell cycle using transcriptional profiling, real time PCR and siRNA interference which will further help us to understand the biological function of PDEF. Our results as well as the critical roles of other Ets factors in cellular differentiation and tumorigenesis strongly suggest that PDEF is an important regulator of prostate gland development and plays a role in prostate and breast cancer progression or development. The new data have further strengthened our believal that PDEF is a prime target for drug development.

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